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Evaluation of Procedures to Desorb Bacteria from Granular Activated Carbon

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16. ABSTRACT

Physical, chemical, and enzymatic means for the desorption of microorganisms from granular activated carbon (GAC) were assessed. Data indicate that homogenization at 16,000 rpm for 3 min at 4°C with a mixture of peptone (0.01%), Zwittergent 3-12 (10^{-6} M), ethyleneglycol-bis-(β amino-ethyl ether)-N,N¹-tetra acetic acid (EGTA 10^{-3} M), and Tris buffer (10^{-1} M, pH 7.0) was effective in desorbing both HPC and coliform bacteria from GAC particles. This method was also effective in removing Escherichia coli from GAC. Four media were tested for their ability to enumerate desorbed HPC bacteria. R2A medium incubated at 18°C for 7 days provided maximal counts.

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Evaluation of procedures to desorb bacteria from granular activated carbon

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Summary

Physical, chemical, and enzymatic means for the desorption of microorganisms from granular activated carbon (GAC) were assessed. Homogenization at 16 000 rpm (4°C) with a solution of Tris buffer (0.01 M, pH 7.0), Zwittergent 3-12 (10^{-6} M), ethyleneglycol-bis-(β amino-ethyl ether)-*N*,*N*'-tetra acetic acid (EGTA, 10^{-3} M) and peptone (0.01%) gave the highest removal efficiency of heterotrophic plate count (HPC) organisms. This method was also effective in removing *Escherichia coli* from GAC. Four media were tested for their ability to enumerate desorbed HPC bacteria. R2A medium incubated at 28°C for 7 days provided maximal counts.

Key words: *Bacterial desorption – Carbon – Procedure evaluation*

Introduction

The basic premise of viable plate count procedures is that one colony arises from a single monodisperse microorganism [1]. This assumption is especially untrue when water contains suspended particulate material. Ridgway and Olson [2] reported that 17% of the particles in the drinking water samples they studied contained attached microbes averaging between 10 and 100 bacteria per particle. The authors concluded that current standard plate count procedures underestimate the actual number of microorganisms by a factor of 1 500- to 15 000-fold. Similar results have been obtained by other investigators [3–5]. Bacteria may be attached to clay, silt, organic and inorganic matter, nematodes, crustaceans, plankton and other microscopic organisms [3–6]. LeChevallier et al. [5] have shown that suspended particulates interfere with microbiological methods; decreasing both the accuracy and reliability of the membrane filter technique.

Since the 1930s, powdered activated carbon (PAC) or granular activated carbon

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(GAC) have been widely used by water treatment plants in the U.S. to combat taste, odor and color problems in surface waters caused by wastes from industrial, municipal and agricultural sources, as well as decaying vegetation and algal blooms [7–11]. Activated carbon is very effective in adsorbing a wide range of organic compounds which provide nutrients for bacterial attachment and growth on these particles [10–15]. Since carbon particles may be densely colonized [15], any particulate breakthrough into finished drinking water will result in a gross underestimation of the true number of bacteria present.

Much of the problem related to the enumeration of particle-associated bacteria arises from a lack of adequate techniques to desorb viable organisms so standard plating procedures can be used. Parsons [21] obtained highest recovery of bacteria from GAC particles using sonication (20 kHz, 180 W output for 4 min). Ashidate and Geldreich (unpublished results), however, found this procedure caused an abrupt decrease in bacterial counts after 3 min. They recommended blending in a Waring blender for 3 min at maximum speed. This procedure was twice as effective as sonication and more than six times as effective as hand shaking for the release of attached organisms.

In this report, we present a modified blending procedure to remove bacteria from activated surfaces. Homogenization of carbon particles at 16 000 rpm (4°C) with a mixture of Zwittergent 3-12 (10^{-6} M), EGTA (10^{-3} M), Tris buffer (0.01 M, pH 7.0) and peptone (0.01%) gave the highest recovery of plate count bacteria. When this technique was applied to drinking water treated with carbon, as much as a 50-fold increase in heterotrophic plate count (HPC) bacteria and a 1200-fold increase in coliform counts were observed when compared to hand shaken samples.

Materials and Methods

Sample sources

GAC populated with indigenous HPC organisms was supplied by municipal drinking water treatment plants located in Pennsylvania (53 million gallons per day (MGD), pop. 480 000), Tennessee (72 MGD, pop. 240 000) and Indiana (6.9 MGD, pop. 46 000). Carbon cores were removed from operating GAC beds, packed in sterile containers, and shipped on ice via overnight delivery to the laboratory for analysis. Tests were performed within 30 h after samples were received.

A source of GAC characterized by a mature biofilm, was also maintained in the laboratory (column 101). Colonized GAC from field samples was mixed and packed in a 6″×2″ diameter plexiglas tube. Chlorinated drinking water (temperature 10°C, free chlorine residual 1.2 mg/l, total organic carbon 4 mg/l) was flowed through the column at a rate of 0.5–1.0 gal/sq ft carbon/min. Effluents from the filter had no detectable free or combined chlorine residual. GAC volume was maintained by addition of colonized carbon when samples were removed.

A separate GAC column colonized with a pure culture of *E. coli* was prepared to allow testing of desorption methods on particles populated with high densities of coliforms. Sterile river water which was inoculated with ca. 10^8 cells/ml of *E. coli* was continuously circulated through a sterile GAC column in a closed system by means of a peristaltic pump (Harvard Apparatus Co., Millis, MA). A flow rate of 27 ml/min was

maintained. Samples were incubated at room temperature and examined after 4 days.

Because of the variety of carbon samples used, a great deal of variation occurred when absorbed populations were enumerated. Individual carbon particles from the same source exhibited varying degrees of colonization as determined by scanning electron microscopy. Replicate samples performed at the same time exhibited up to 36% variation.

Nuchar GAC (8×30 mesh, Westvaco Chemical Division, Carbon Department, Covington, VA), was used when known attached populations were desired. Powdered activated carbon (PAC) was created by grinding GAC. Both were oven sterilized at 180°C for 3 h.

Microbiological analyses

HPC organisms

Enumerations were performed by the spread plate technique in triplicate on plate count agar (Difco Laboratories, Detroit, MI) and incubated at 35°C for 2 days. Typically, 0.1 g of colonized carbon was homogenized. Media comparisons utilized R2A [16], mSPC, [17], 0.1% strength plate count agar (abbreviated 0.1 SPC; formulated from plate count agar, Difco) and plate count agar (abbreviated SPC) all incubated at 28°C for 2–7 days.

Coliform analyses

Escherichia coli isolated from the East Gallatin River was maintained on stock culture agar (Difco). Cells were grown in TLY broth [18] for 18–24 h at 35°C. Bacteria were added to 100 ml cold (4°C) sterile Milli-Q reagent grade water (Millipore Corp., Bedford, MA) containing 1.0 g sterile PAC to a final concentration of approximately 10^6/ml. After 20 min the supernatant was decanted and a plate count performed to enumerate unattached cells. The carbon was gently washed twice with cold sterile reagent grade water to remove any traces of supernatant. This procedure was employed when a known amount of attached bacteria was desired. The attachment time of 20 min was selected to optimize exposure of the bacteria to the carbon and minimize endogenous growth. Previous unpublished experiments determined that nearly 100% of the added cells became attached to PAC during this time.

Organisms were enumerated in triplicate by the spread plate technique on m-Endo (Difco) and m-T7 [19] agar media. Both media were incubated for 20±2 h at 35°C. Injury is described as the percent difference between counts on m-Endo and m-T7 media [19].

Homogenization technique

Equipment

Physical desorption experiments utilized a Biosonik IV sonicator (DuPont/Sorvall, Newtown, CT), Waring blender (Model #700) and Omni-mixer homogenizer (DuPont/Sorvall; model #17150). All sample containers were autoclaved at 15 lb pressure for 20 min to insure sterility. Carbon (0.1 g) was weighed aseptically and processed in

100 ml cold, sterile reagent grade water. Appropriate sterility controls were also performed.

Chemicals and enzymes

Zwittergent 3-12 and Chapso were obtained from Calbiochem-Behring Corp (LaJolla, CA). Peptone, gelatin, tryptone and beef extract solutions were made using Difco products. Tergitol 7 was manufactured by the J.T. Baker Chem. Co. (Phillipsburg, NJ). Tris buffer was purchased from the G. Fredrick Smith Chem. Co. (Columbus, MO). All other chemicals and enzymes were obtained from the Sigma Chemical Co. (St. Louis, MO), and stored according to manufacturer's recommendations.

Results

In order to accurately enumerate bacteria when particles were present, several methods of desorption and enumeration were evaluated. These included physical, chemical and enzymatic desorption procedures as well as the determination of optimal growth conditions of the desorbed bacteria.

Physical parameters

Initial experiments compared the efficiency of three methods to physically strip bacteria from a particle surface. Homogenization and blending act by generating shear forces, and sonication may act by disrupting the cell-particle bond. In these evaluations it was important to know whether the treatment was lethal to bacteria. To evaluate the survival of treated cells, control experiments using known concentrations of HPC organisms or *E. coli* were suspended in sterile surface water. Figure 1 illustrates the effect of homogenization and blending on viable HPC organism enumerations. Temperature change of the cell suspension is also presented. Use of the Waring blender produced a rapid temperature increase with 30°C attained following 3 min of blending and 50°C

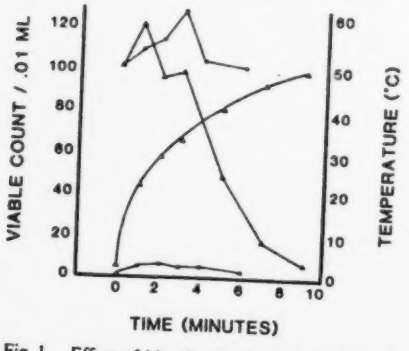

Fig. 1. Effect of blending or homogenization on heterotrophic plate count viability. (●) homogenizer viable count, (▲) Waring blender viable count, (○) homogenizer temperature, (△) Waring blender temperature.

after 10 min. Elevated temperatures corresponded with decreased bacterial viability. The structure of the homogenizer permitted immersion of the blending vessel in an ice bath. Temperature of the contents was maintained near 4°C. Viable counts increased from 100 to 120 cfu/0.1 ml with 3 min of homogenization, presumably from the dispersion of bacterial clumps.

E. coli counts did not decrease during blending until 45°C was exceeded, but some injury (ca. 60%) did result. Cell numbers remained constant and no injury was observed when *E. coli* was homogenized at 4°C.

Sonication has been suggested as a possible desorption method in other studies [11]. The sonicator used in these experiments killed *E. coli* cells at minimum, 30, 50 and 75% of maximum power settings, even when the sample was iced (74% mean decrease in cfu/ml in 9 min). Cells did not become injured before death occurred. HPC organisms were also enumerated at a reduced level. Sonication may have removed bacteria from particle surfaces, but the number recovered was underestimated due to death of the cells.

Homogenization for 3 min at 16 000 rpm was selected as the best physical means of removing organisms from particles and protecting viability once the cells had been desorbed.

Chemical and enzymatic parameters

The release of cells from carbon particles by the addition of certain chemicals acting in cooperation with homogenization was examined. Since it was anticipated that a variety of bacteria would have different attachment mechanisms, 25 chemicals, combinations or concentrations were selected and tested on colonized GAC. It was proposed that these chemicals could remove binding materials, negate surface charges, protect the cells, and allow shear forces to act more efficiently.

When known numbers of *E. coli* were attached to PAC and homogenized only 4% of the population was recovered (data not shown). It was assumed that the organisms were reattaching to new reactive sites on the carbon surface before enumeration could be performed. Compounds that could block this reabsorption were tested in several concentrations. These included peptone, beef extract, gelatin and tryptone at concentrations of 10, 1 and 0.1 g/l. Peptone resulted in the greatest recovery of organisms (83–94% of added attached population) and a concentration of 1 g/l demonstrated only a small amount of foaming when the samples were homogenized (data not shown).

Two zwitterionic detergents (Zwittergent 3-12 and Chapso) were selected to determine if surfactants could improve bacterial desorption. Several concentrations of each compound were evaluated for varying lengths of homogenization time using GAC colonized with HPC organisms. Zwittergent 3-12 had the greatest desorption efficiency at 10^{-6} M and 3 min of homogenization (143% of control value) (Fig. 2). Chapso was not effective in the concentrations tested.

Attachment of cells to GAC can be attributed to properties other than charge, especially when mature biofilms have been established. Eight chemicals capable of altering other possible bonding mechanisms were selected and tested at several different concentrations alone and in combination with Zwittergent 3-12 to determine their ability to

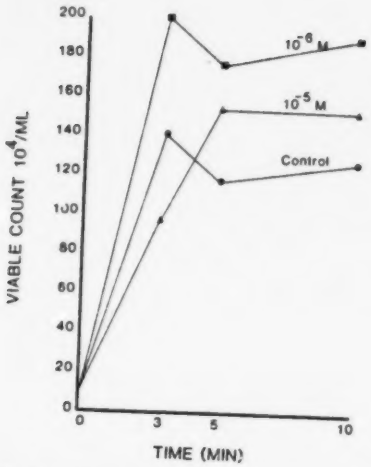

Fig. 2. Effect of homogenization of colonized GAC in the presence of Zwittergent 3-12. Counts are of cells released into the homogenization solution. (■) 10^{-6} M, (▲) 10^{-5} M, (●) control; reagent grade water.

TABLE I

COMPOUNDS USED TO DESORB HETEROTROPHIC PLATE COUNT BACTERIA FROM GRANULAR ACTIVATED CARBON BY HOMOGENIZATION

Values are the mean of four experiments and represent the rates of cfu/ ml in the experimental technique compared to a control treatment (homogenization with 10^{-6} M Zwittergent 3-12 only). SD = standard deviation. + or - indicates whether compound was used with or without Zwittergent 3-12

Compare	Zwittergent 3-12	$\frac{X}{\text{Zwittergent 3-12}} \pm$ S.D.
Tris buffer (0.01 M, pH 7.0)	+	1.26 ± 0.18
0.85% Saline	+	0.94 ± 0.36
2.0% Saline	+	0.65 ± 0.14
5.0% Saline	+	0.48 ± 0.35
0.1% Tween 80	+	1.28 ± 0.32
0.01% Tween 80	+	1.19 ± 0.32
0.05% EDTA	+	0.93 ± 0.47
0.05% EDTA	-	0.90 ± 0.28
0.005% EDTA	-	0.69 ± 0.06
0.005% EDTA	+	1.21 ± 0.48
1 mM EGTA	+	1.14 ± 0.23
1 mM EGTA	-	1.36 ± 0.66
0.001% Triton X-100	+	1.11 ± 0.08
0.001% Tergitol 7	+	1.27 ± 0.26
10^{-4}% Tergitol 7	+	0.98 ± 0.12
0.1% Tergitol 8	+	0.55 ± 0.05
0.01% Tergitol 8	+	1.04 ± 0.33
0.001% Tergitol 8	+	1.09 ± 0.18
0.1% Peptone	+	1.11 ± 0.09

increase HPC bacterial removal during homogenization (Table 1). Of the compounds tested, EGTA, Tween 80, buffer and 0.001% Tergitol 7 provided greater mean values than the control. Standard deviations indicated that these treatments were not significantly different. Due to the high degree of variability in colonization of particles, the mean values were selected as the most appropriate measure of treatment effectiveness. Various combinations of these chemicals were further tested (see below).

Several reports have shown that extracellular polymers are involved in the attachment of bacteria to surfaces [10, 15, 21]. Six enzymes or reactive compounds at ten concentrations were examined for their potential to disrupt polymer material. The enzymes tested were trypsin, pepsin, amylase, protease, carboxyl esterase and papain. Sodium-M-periodate was also evaluated. Initially, these compounds were allowed to react with colonized GAC for lengths of time varying up to 30 min prior to homogenization. Five min of contact time produced the greatest recovery of organisms with all compounds except amylase which was most effective if added just prior to homogenization (data not shown). Subsequent experiments examined the desorption efficiency of these compounds at various concentrations and optimal contact times when compared to a Zwittergent 3-12 homogenized control. The results indicated that the use of trypsin and amylase consistently yielded counts near or higher than the Zwittergent 3-12 control (1.10–1.34- and 0.98–1.60-times the control, respectively). Trypsin produced optimal results at 1 g/l and amylase performed best at 10 mg/l.

Based on the above experiments, a number of homogenization mixtures were developed employing each compound at its optimum concentration and reaction time. The desorption efficiency of ten formulations for removing HPC organisms from colonized GAC was determined. These consisted of combinations of Tris buffer (0.01 M, pH 7.0), peptone (0.01%), Zwittergent 3-12 (10^{-6} M), EGTA (10^{-3} M), trypsin (1 μg/l), amylase (10 mg/l) and Tergitol 7 (0.001%). Carbon samples tested included GAC cores from drinking water treatment plants populated with indigenous HPC bacteria. Considerable variation in results for each technique was obtained. Differences between homogenization techniques are not statistically significant, but overall trends can be evaluated. Homogenization at 4°C for 3 min with buffer (Tris, 0.01 M, pH 7.0), peptone (0.01%) and Zwittergent 3-12 (10^{-6} M) produced the highest average result (1.09-times that with Zwittergent 3-12 and peptone alone). Enzymes did not appreciably increase bacterial counts.

Additional experiments were performed on thirteen GAC core samples using the five consistently best homogenization formulations as determined above (Table 2). All exhibited a pronounced improvement over those shaken by hand (average increase of 1.43 log). Homogenization with mixture #4, consisting of buffer (Tris 0.01 M, pH 7.0), peptone (0.01%), Zwittergent 3-12 (10^{-6} M) and EGTA (10^{-3} M), although not statistically higher, resulted in the highest mean recovery of HPC organisms (2.56 logs higher than hand shaken).

Carbon particles colonized in the laboratory with known bacterial levels were used to confirm the effectiveness of the homogenization procedure. Sterile virgin PAC (0.1 g) was exposed to a suspension of 10^6 *E. coli* per ml for 20 min. Virtually all of the bacteria became attached to the carbon during the contact period as determined by a plate count of the suspending water. After desorption by homogenization at 16 000 rpm

TABLE 2

EFFECT OF VARIOUS HOMOGENIZATION TECHNIQUES FOR THE DESORPTION OF HETEROTROPHIC PLATE COUNT BACTERIA FROM GRANULAR ACTIVATED CARBON

Carbon source	Homogenization technique #					
	1	2	3	4	5	6
Column 101	0.005[a]	1.00	0.56	nd	0.65	0.75
Column 101	0.03	1.00	1.12	nd	0.70	0.78
Column 101	0.32	1.00	1.24	nd	0.72	0.86
Tennessee	0.07	1.00	1.53	1.87	1.07	nd
Indiana	0.06	1.00	nd[b]	0.75	nd	0.74
Pennsylvania #31	0.05	1.00	1.00	0.36	0.38	nd
Pennsylvania #35	0.12	1.00	1.82	3.06	1.31	1.41
Pennsylvania #3	0.04	1.00	1.70	0.64	0.58	0.32
Pennsylvania #15	0.009	1.00	1.02	2.56	1.48	1.00
Pennsylvania #15-A	0.10	1.00	1.71	1.41	nd	nd
Pennsylvania #15-B	0.01	1.00	0.98	1.10	1.06	nd
Pennsylvania #15-A	0.002	1.00	1.82	2.30	0.91	1.46
Pennsylvania #15-B	0.03	1.00	0.71	0.56	0.75	0.69
Mean ± S.D.	0.07 ± 0.08	1.00	1.27 ± 0.44	1.46 ± 0.94	0.87 ± 0.33	0.89 ± 0.36

[a] Values represent the ratio of cfu/ml of the experimental treatment compared to a control treatment (#2).
[b] nd – not done.
#1, hand shaken with Tris buffer (pH 7.0, 0.01 M) and peptone (0.01%).
#2, blended with Tris buffer and peptone.
#3, #2 plus Zwittergent 3-12 (10^{-6} M).
#4, #2 plus Zwittergent 3-12 and EGTA (10^{-3} M).
#5, #2 plus Zwittergent 3-12 and Trypsin (1 g/l).
#6, #2 plus Zwittergent 3-12, EGTA and trypsin.

for 3 min with a solution of buffer (Tris, 0.01 M, pH 7.0), peptone (0.01%), Zwittergent 3-12 (10^{-6} M) and EGTA (10^{-3} M), approximately 90% of the known bacterial population was recovered as determined by plate counts. Carbon particles were also observed using acridine orange direct count epifluorescence microscopy before and after homogenization. Most of the carbon particles were free of bacteria.

Enumeration of heterotrophic plate count organism

Determination of optimal conditions for desorption of microorganisms from GAC was not the sole consideration for maximal bacterial enumeration. The medium and growth conditions under which these cells were enumerated was of equal importance. Four media (SPC, 0.01 SPC, mSPC, and R2A) were evaluated with various incubation times at 28°C to determine which combination provided the greatest enumeration of desorbed HPC bacteria (Fig. 3). Although the results obtained using R2A were lower than those with SPC or 0.1 SPC numbers at 2 days of incubation, by day 3 it surpassed all other media. At day 7, R2A enumerated 184-times the bacteria found on mSPC.

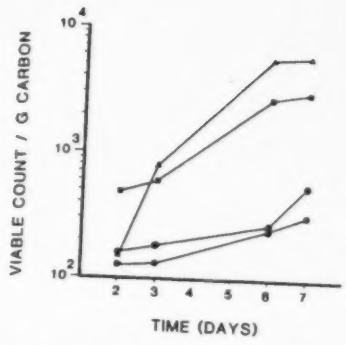

Fig. 3. Comparison of enumeration efficiency following desorption from GAC. Various media used; R2A (▲), 0.1 SPC (■), SPC (●), and mSPC (⬮) all incubated at 28°C.

TABLE 3

COMPARISON OF VARIOUS HOMOGENIZATION TECHNIQUES FOR THE DESORPTION OF *E. COLI* FROM GRANULAR ACTIVATED CARBON

Date	Homogenization technique #								
	2	3	4	5	6	8	11	12	13
7-28-83	2.78[a]	2.00	2.22	2.11	3.11	2.06	1.56	1.22	1.00
8- 3-83	3.05	2.37	2.63	2.47	2.73	2.47	2.84	1.26	1.00
8-31-83	1.96	1.16	1.07	1.56	1.38	0.95	1.43	0.65	1.00
9-20-83	1.82	2.27	2.36	4.29	2.25	1.53	nd[b]	0.50	1.00
9-28-83	1.62	3.46	1.38	2.46	0.62	2.15	1.62	0.31	1.00
10- 5-83	2.20	2.60	2.70	3.80	2.40	3.80	1.90	0.80	1.00
Mean ± S.D.	2.24 ± 0.56	2.31 ± 0.75	2.06 ± 0.68	2.78 ± 1.04	2.08 ± 0.92	2.16 ± 0.97	1.87 ± 0.57	0.79 ± 0.38	1.00

[a] Values represent the ratio of cfu/ ml of the experimental treatment compared to a control treatment (#13). All counts done on m-Endo agar.

[b] nd – not done.

2, blended with Tris buffer (pH 7.0, 0.01 M) and peptone (0.01%).

3, #2 plus Zwittergent 3-12 (10^{-6} M).

4, #3 plus EGTA (10^{-3} M).

5, #3 plus Trypsin (1 μg/l).

6, #3 plus EGTA and trypsin.

8, #3 plus EGTA, trypsin and amylase (10 mg/l).

#11, #3 plus amylase.

#12, hand shaken, no additives.

#13, blended, no additives.

The highest initial number was provided by 0.1 SPC, but by day 7 this was 60% of the R2A value. With the exception of SPC medium, counts did not increase appreciably between days 6 and 7. R2A medium incubated for 7 days at 28°C was selected as the medium, time and temperature of choice.

The five best homogenization formulations previously described were retested with R2A medium incubated at 28°C for 7 days. Once again, homogenization with buffer (Tris, 0.01 M, pH 7), peptone (0.01%), Zwittergent 3-12 (10^{-6} M) and EGTA (10^{-3} M) provided the highest recovery (data not shown).

Coliform enumerations

It was unknown whether the desorption procedures would injure coliforms and therefore decrease the sensitivity of the proposed method. Experiments were conducted using m-T7 and m-Endo media to enumerate *E. coli* that had been desorbed by homogenization from GAC. The amount of injury (measured as the difference between the two media) did not appreciably increase with any of the homogenization techniques.

In addition, the ability of several homogenization mixtures to desorb *E. coli* grown on GAC was determined (Table 3). Several techniques [2–4, 6] (see Table 3) showed results at least 2-fold higher than homogenization with no additives. Due to the high variability among carbon samples there was no significant difference between any of the five formulations. Since buffer (Tris 0.01 M, pH 7.0), peptone (0.01%), Zwittergent 3-12 (10^{-6} M) and EGTA (10^{-3} M) had consistently desorbed more HPC bacteria, it was chosen as the best method when mixed populations of HPC organisms and coliforms were expected on GAC field samples.

Discussion

Documentation of bacterial numbers in carbon-treated effluents is difficult to compare since a variety of microbiological methods have been used. Parsons [21] suggested sonication as a means of removing bacteria from GAC particles, but our results and those of Ashidate and Geldreich (unpublished results) found that sonication killed bacteria once they were desorbed from the carbon surface. These researchers recommended blending GAC samples in a Waring blender for 3 min of maximum speed. Data presented in this report indicate that homogenization at 16 000 rpm for 3 min at 4°C with a mixture of peptone (0.01%), Zwittergent 3-12 (10^{-6} M), EGTA (10^{-3} M) and Tris buffer (10^{-1} M, pH 7.0) was effective in desorbing both HPC and coliform bacteria from GAC particles. The effectiveness of the desorption technique is evidenced by recovery of 90% of the seeded bacterial populations. In addition, observations made by scanning electron microscopy and acridine orange direct counts revealed that homogenized particles were virtually bacteria free (data not shown). In on-going experiments where this desorption technique has been applied to particles from carbon treated drinking water, increases as high as 50-fold for HPC bacteria and 1200-fold for coliform organisms over conventional hand shaken enumerations have been obtained.

A number of investigators have discussed methods to enumerate HPC bacteria in carbon treated drinking water. While a variety of procedures have been used, generally

low viable counts (50 cfu/ml) were reported when low temperatures and short times of incubation were employed (2–3 days at 20–22°C) [8, 10]. Higher bacterial densities (500–5000 cfu/ml) were reported emanating from carbon filters when longer incubations were used (5–14 days) [8, 12, 14, 21]. Our results agree with others [11] who have shown that the highest HPC densities are obtained with R2A medium incubated at 28°C for 7 days.

Whereas desorption studies have been performed in other disciplines of microbiology [22–24], little attention has been given to improving the accuracy of enumerating viable cells in potable waters containing particulate carbon. Results presented in this report demonstrate an effective procedure for desorbing both HPC and coliform bacteria from GAC particles.

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